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Zinc and manganese effect seed quality and germination in common bean (*Phaseolus vulgaris* L.)

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Abstract: Zinc (Zn) and manganese (Mn) are important micronutrients in plant growth and development. In this study, the effect of Zn and Mn sulfate treatments at different growth stages of mother plants in common bean on seed production and germination potential was carried out. Results showed that zinc sulfate ($ZnSO_4$) treatments significantly affected yield and pod number per plant compared to manganese sulfate tetrahydrate ($MnSO_4$) treatments. Treatment in any form or at any stage of growth increased yield and its components in common bean. The highest efficiency of $ZnSO_4$ was obtained in the pod production stage; $MnSO_4$ enjoyed greater efficiency in the flowering stage; and the minimum efficiency was observed for soil application. The zinc and manganese content in the seed production by foliar application in the stem elongation + flowering + pod production stages were the highest. The two-year performance increase for three-stage consumption of zinc sulfate in comparison to no-consumption and soil consumption was respectively 173 and 202 g/m²; by using manganese sulfate, the consumption was 92 and 134 g/m². This increased yield seems economical compared with the costs incurred in the preparation and consumption of it. The increase in seed quality due to the increasing content of micronutrients, especially zinc, was noticeable. The results of the germination experiment showed that the plant dry weight and germination percentage treated with $ZnSO_4$ in the lab were significantly higher than $MnSO_4$. In the treatments by foliar application, the plant height was related to the seeds treated with Zn fertilizer, but in soil treatments, the use or nonuse of Mn fertilizer caused higher plant height. The seed production from the treated crops with foliar application at flowering + stem elongation germination was faster than the other treatments, but the minimum rate was obtained for the control group. The result showed that Zn and Mn-sprayed seeds had a significant impact on the vigor of the seedlings.

Key words: Common bean, micronutrients, foliar application, vigor

1. Introduction

Phaseolus vulgaris L., also known as the common bean, is one of the important crops in the legume family and is grown worldwide for its edible dry seeds or unripe fruit (fresh pods). The plant is grown in temperate zones as well as in temperate regions within the subtropics. As a dry seed, it is an important crop in Africa and in Central and South America. Many bean types are cultivated that exhibit vast differences in seed coat coloration and pod characteristics (Koksal et al., 2021; Nadeem et al., 2021; Kul, 2022). Currently, the common bean is one of the main sources of protein and calories in the human diet, and because of the importance of this crop, its cultivation in the world is increasing steadily. The low solubility of elements in alkaline pH, the presence of carbonate and bicarbonate ions in irrigation water, and a high intake of

phosphorus have caused a deficiency of micronutrients, especially Zn, Mn, Fe, and B. The intake of these elements, especially Fe, and micronutrients, especially Zn, Mn, Fe, and B. During the intake of these elements, especially Fe in the cell sap, they are deposited in the vessels and do not reach the other parts of the plant. Because of these deficiencies, the average yield in different crops is generally low (Altındag et al., 2006; Ziaeiian and Malakouti, 2001; Kumar et al., 2022; Alzuabir, 2023). Factors such as soil reaction, calcareous rate, and amount of organic matter affect the availability of nutrients (Rashid and Ryan, 2004; Öktüren Asri, 2022). The foliar application eliminates the problem of limited absorption of nutrients in the soil. Foliar application of Fe, Mn, and Cu may be more effective than soil application because, in soil application, the particles absorb nutrients, making them less available for

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the root system (Roosta et al., 2013). Mn and Zn are among the essential elements needed by crops; a lack of them severely reduces crop yield and sometimes even causes them to perish. Manganese has an important role in a number of cellular activities, such as the stability of protein structure, chloroplast structure, and photosynthesis. If this element is used too much, it becomes toxic for the plant (Aref, 2011). Among the micronutrients, Zn deficiency is the most common one, and this element is the most important micronutrient for crop growth and reproduction (Rashid and Ryan, 2004; Obrador et al., 2007; Dawadi et al., 2022). Zn deficiency is widespread among the crops in the soil of arid and semiarid regions of the world, and it is reported that the growth and performance of crops in these regions strongly decrease (Torun et al., 2003; Younas et al., 2022). Several factors affect the seed vigor, the most important of which are the genetic background, environment and nutrition of the mother crop, seed reserves, the stage of maturity at harvest, and pathogens. The genetic structure of the seed usually has the most effect on its vigor (Boonchuay et al., 2013). Genotype controls the potential and genetic abilities like the growth and germination of the seeds, but due to environmental factors, it cannot exert its full effect (Copeland McDonald, 2001; Reed et al., 2022). Adverse environmental conditions bring about indirect harmful effects on the processes of seedling and seed yield, which often appear after harvest. The concentration of the production of the seeds of some agricultural products in particular regions implies the effect of environmental factors on the development and quality of the seeds (Copeland and McDonald, 2001; Abbas et al., 2022). By using enriched seeds with micronutrients, the obtained plants have better germination and primary growth. The effect of mother plant nutrition on seed production has been examined in some of the studies. Maheshbabu et al. (2008) and Chen et al. (2022) reported that the application of fertilizers containing high consumption and micronutrients significantly increased the seed quality parameters such as germination, root, and shoot length, the dry weight of the seedlings, and decreased the electrical conductivity of soybean seeds. The percentage of germination, root and shoot length, and the strength index of okra (*Hibiscus esculentus* L) as a result of fertilizer treatment as well as $MnSO_4$ (50 kg/ha) increased. The improvement in seed quality was attributed to Mn application, which improved the photosynthetic capacity of the crop (Karibasappa et al., 2007). Devi et al. (2003), in an experiment on Indian mustard, concluded that seed size could affect the strength of the crops, and the crops treated with micronutrients of Fe, Zn, Cu, and Mn had more weight and higher vigor. The application of Mn significantly increased the dry matter in bean and corn. The Mn application significantly increased its intake in bean and corn (Fageria et al., 2007).

Researchers reported that foliar applications with $MnSO_4$ increased the net assimilation rate, seed yield, and crude protein content of corn (Thalooth et al., 2006). The use of Mn fertilizer strips adjacent (rates of 0.34 to 1.34 kg/ha) to the maize led to a seed yield resulting from spraying Zn fertilizer on the soil surface at a rate of 26.9 kg/ha (Fageria et al., 2007; Gadallah et al., 2022). Another study on rice showed higher effectiveness of the seed-coated application than soil application. The total dry matter and grain yield of rice were not affected by the use of different methods (soil application and seed coat) (Sultana et al., 2001). Zn spraying affected the weight of the lentil 100 seeds, leading to a higher yield. The response of lentils to the application of these elements may be due to a lack of nutrients in the soil. The amount of these elements in lentils improved as a result of using Zn (Zeidan et al., 2006). Rice yield in dry land conditions and oxisol soils in Brazil increased by using Zn. With an increase in the concentrations of Zn in the soil, the absorption of Zn in rice and common beans increased (Fageria et al., 2007). Subramonian et al. (2006), in their study on grapevine, reported that because the transfer of Zn from the sprayed leaves onto the nonsprayed leaves is very low, foliar application must be done in several stages. Salamatbakhsh et al. (2012), in their study on bean, reported that foliar application led to the highest concentrations of Fe, Zn, and Mn in the leaves in comparison to soil application. In some field studies, the number of seeds per pod was affected by foliar application of Mn and Zn, and the effect of Mn was higher than the others. However, foliar application of Mn and Zn did not significantly affect the 100 seed weight. The height of the plant was affected by the foliar application of Zn and Mn, and this effect was more visible for Zn. This result was expected because Zn is an essential element for the synthesis of tryptophan, which expedites growth. The application of Zn increased the green bean yield by up to 24% (Teixeira et al., 2004; Jalal et al., 2022). The researchers reported that foliar application of green beans with Mn increased the dry weight compared to the control group. Madani et al. (2007) showed that the application of 40 kg/ha Zn in the form of soil and consequently foliar application before flowering and during seed filling in soybeans significantly increased the quantity of soybean yield. According to Ozbahce and Zengin (2014), the fertilizer ME-FA sprayed significantly increased the greatest yields of common beans (243.80 and 257.74 kg/d⁻¹). The MS-SA treatments produced the lowest yields for both years (166.97 and 180.60 kg da⁻¹). According to Boonchuay et al. (2013), foliar zinc only raised the concentration of zinc in paddy soil after flowering, and the increases were greater with subsequent applications. The husk showed the biggest increases in zinc content—up to a tenfold increase—while brown rice showed lesser increases. Seedlings from seeds containing 42 to 67 mg of zinc per kg exhibited longer

roots and coleoptiles than seedlings from seeds containing 18 mg of zinc per kg — an effect that subsided after a few days of germination. Rice grains' zinc content can be efficiently increased. Gopal and Nautiyal (2012) revealed that the poor growth of plants grown in soil without Zn applications (0 mg Zn kg^{-1}) was improved by applications of Zn (20 mg Zn kg^{-1}) more when Zn was applied with two foliar sprays. Application of Zn (20 mg Zn kg^{-1}) with two foliar sprays also proved beneficial for maximizing Zn concentrations in grains and other plant parts. The wheat varieties NW 1076, K 3827, NW 2036, and UP 262 appeared highly responsive to the treatments. The aim of this study was to evaluate the effects of Zn and Mn in the production period of the mother plant on the production and seed germination of common bean cv. Khomein.

2. Materials and Methods

2.1. Plant material and location of experiment: This experiment was conducted in two consecutive agricultural years of 2016 and 2017 in the College of Agriculture of Islamic Azad University, Tabriz Branch. In April of both years, a number of soil samples were obtained from several parts of a field at a depth of 0–30 cm and sent to the soil physics laboratory. The results are presented in Table 1. The rate of Mn and Zn micronutrients based on the critical level of micronutrient elements (Table 2) in the agricultural soil of the region was low to medium, justifying its consumption in the study.

The present study was conducted in the field in two agricultural years, using a factorial experiment based on the randomized complete block design with three repetitions.

2.2. Experiment treatments

The experimental factors involved in the study included: 1) micronutrient fertilizer at two levels of ZnSO_4 and MnSO_4 , 2) different applications, and the time of application of different micronutrient fertilizers at nine levels: control (A_0), soil consumption (A_1), foliar application at stem elongation (A_2), foliar application at flowering stage (A_3), foliar application at pod production (A_4), foliar application at stem elongation + flowering (A_5), foliar application at stem elongation + pod production (A_6), foliar application at flowering + pod production (A_7), and foliar application at the flowering + stem elongation pod production (A_8).

2.3. The field conditions

The field consisted of 54 plots, each having 4 rows of 50 cm furrows. The Khomein variety of common bean seeds was planted at a distance of 5 cm from each other. In soil treatments, the micronutrient fertilizers, on the basis of the amount of micronutrients in 30 kg/ha ZnSO_4 and 25 kg/ha MnSO_4 , were consumed simultaneously with planting. After planting and plant establishment in the 2–4 leaf stage, thinning and weeding operations were done. In thinning operations, the distance of the plants increased from 5 cm to 10 cm. The irrigation of the plots was uniform, and the average was once every 7 days.

Table 1. Physical and chemical condition of the soil samples from field crops in two years.

| Clay (%) | Silt | Sand | Mn (ppm) | Zn (ppm) | k (ppm) | p (ppm) | EC T.N% | pH (ds/m) | Depth sampling (cm) | Year | |
|-------------|------|------|-------------|-------------|------------|------------|------------|--------------|---------------------------|------|------|
| 14 | 20 | 66 | 1.64 | 1.42 | 1120 | 12 | 0.138 | 1.8 | 7.67 | 0–30 | 2016 |
| 13 | 19 | 68 | 3.04 | 1.12 | 571 | 80 | 0.113 | 2.05 | 7.70 | 0–30 | 2017 |

Table 2. The critical element micronutrients in agricultural soils (Ziaieian and Malakouti, 2001).

| Element | Measure | The amount of soil (mg/kg) |
|-------------------|---------|----------------------------|
| Mn by DTPA method | low | <5 |
| | average | 9–12 |
| | high | >30 |
| Zn by DTPA method | low | <0.5 |
| | average | 1.1–3.0 |
| | high | >6 |

Foliar application treatments were based on the levels defined in the experiment. Foliar application was done by using a 20-L spray machine with a distance of 30 cm from the plants and at specified periods and stages with a concentration of 6 per mL in the early morning before the field irrigation. The harvesting operations were carried out in September in both the field irrigation. The harvesting operations were carried out in September, in both years after the appearance of signs of ripening. The harvest time of the bean depended on the time of planting and the type of sowing. In Khomein variety, the pods do not ripen at the same time, and harvesting takes place when 65%–75% of the pods ripen. In an area of 1 square m, 10 crops, as a sample of the total population, were selected to determine the number of pods per plant and seeds per pod and to measure the 100 seed weight, seed yield, harvest index, and efficiency of the consumption of micronutrients. The seed yield in this area was calculated by weighing the total number of harvested seeds. To calculate the efficiency of Zn and Mn in the experimental treatment groups, the following equation was used: $E_e = YDE - YDF/F$, in which YDE is the yield per unit with fertilizer application, YDF is the yield per unit control group, and F is the amount of fertilizer (Godrod et al., 1988).

2.4. The Germination experiment

The vigor of the produced seeds in the second year was tested in vitro using a factorial random design with 4

replications involving 72 plots. The obtained seeds were used for testing the vigor of the obtained seeds and determining the content of Zn and Mn. The study method was based on International Safe Transit Association (ISTA) rules and included a standard germination experiment. The seeds from field treatments were put in Petri dishes in a controlled germinator environment at a temperature of 2 ± 20 °C. The count of seed germination, root and shoot length measurements in normal seedlings was done at the end of the tenth day. After the experiment, using the data, the root to shoot ratio, percentage seedling dry weight, the number of seed germinations and germination percentages, and the germination speed and time range were calculated.

2.5. Statistical analysis

After testing the normal distribution of data variances, ANOVA was used for the field study and a simple ANOVA for testing the germination. Analysis of variance and mean comparisons were conducted using MSTATC, and Excel software was used to draw graphs. It should be noted that the comparison of means by Duncan's multiple range was used at a probability level of 5%.

3. Results and discussion

The results of the ANOVA indicated that the effects of the type of micronutrient, the consumption stage, and their interactions were significant for most of the traits (Table 3).

Table 3. Analysis of variance and mean square for studied traits in field experiment (2016–2017).

| S.O.V. | df | MS | | | | | | | |
|--------------------|----|--------------------------|-------------------------|-------------------|-------------|---------------|-----------------------|---------------------|---------------------------|
| | | Number of pods per plant | Number of seeds per pod | 100 kernel weight | Grain yield | Harvest index | Zinc content in grain | Mn content in grain | Micronutrients efficiency |
| Year (Y) | 1 | 8347** | 671** | 254** | 2,874,681* | 2.8* | 96.4** | 41.34** | 38,964* |
| Replication | 4 | 97.1 | 35.4 | 5.5 | 1478924 | 1.3 | 9.8 | 1.22* | 25,503 |
| Fertilizer (F) | 1 | 7588** | 58.7 | 4.7 | 7,824,643** | 1.7 | 8.8 | 1.06 | 27,228 |
| F×Y | 1 | 1107** | 28.4 | 1.8 | 928746 | 1 | 2.6 | 1.09 | 14,144 |
| Spraying Stage (A) | 8 | 936.5** | 581.4** | 12.3* | 3,958,749** | 9.4** | 105.4** | 7.88** | 45,908* |
| A×Y | 8 | 89.7 | 391.4** | 18.9* | 287498 | 4.4** | 54* | 4.89** | 18,291 |
| F×A | 8 | 64.8 | 47.6 | 1.8 | 2,468,935* | 2.3 | 8.05 | 1.12 | 29,569 |
| F×Y×A | 8 | 298 | 25.8 | 0.6 | 357,985 | 1.2 | 7.8 | 1.04 | 30,283 |
| Error | 70 | 99.3 | 19.5 | 0.14 | 238,481 | 1.1 | 2.6 | 0.43 | 27,542 |
| CV (%) | | 11.3 | 18.8 | 13.7 | 22.1 | 11.3 | 18.9 | 26.9 | 15.6 |

* and **: significant at 5% and 1%, respectively

3.1. The number of pods per plant

Comparing the means of the data (Figure 1) revealed that the number of pods per crop by application of ZnSO₄ was higher than that of MnSO₄ in both years. This superiority was 19.44% in the first year and 17.97% in the second year. A significant difference was observed between the use and nonuse of micronutrients (in both soil and foliar application) in the number of pods per crop, and on average, the use of fertilizer resulted in 75.31% more pods. The foliar application crops in flowering + stem elongation + pod production stages (A8), flowering + pod production (A7), and stem elongation + flowering (A5) had the highest number of pods per plant, and soil application had 2.27, 1.33, and 1.42 pods more than the control group with

2.77, 1.83, and 1.92 pods. Other foliar applications were not significantly different from soil application treatments (Figure 2). Applications of nutrient elements, especially Zn, had positive effects on the formation of stamens and pollen grains. Therefore, part of the increase observed in this test is attributed to the effect of micronutrients. In other words, with the increase in the flags' activity, the fertility of the flowers becomes more favorable way, and subsequently, the number of the produced pods for each plant increases. According to Teixeira et al. (2004), Ozbahce and Zengin (2011) reported a 51% increase in the number of pods per plant as a result of using Mn and Zn compared to the control group.

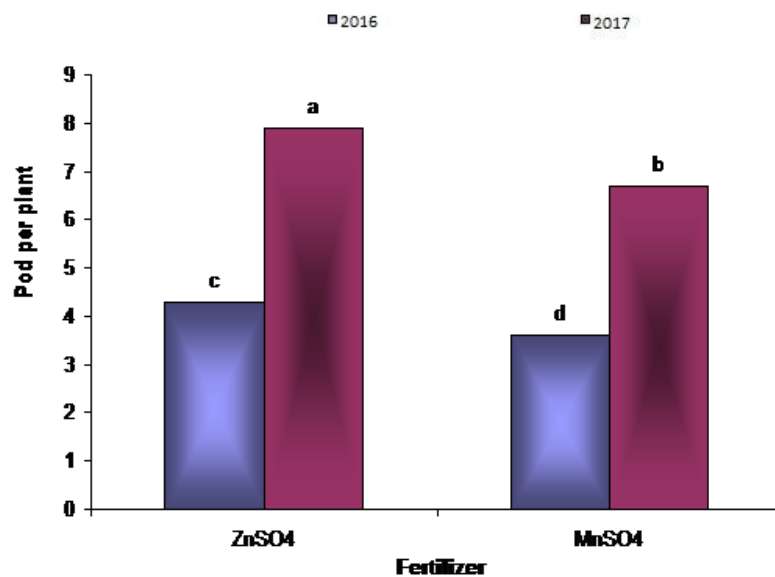


Figure 1. Effects of ZnSO₄ and MnSO₄ on bean pod number.

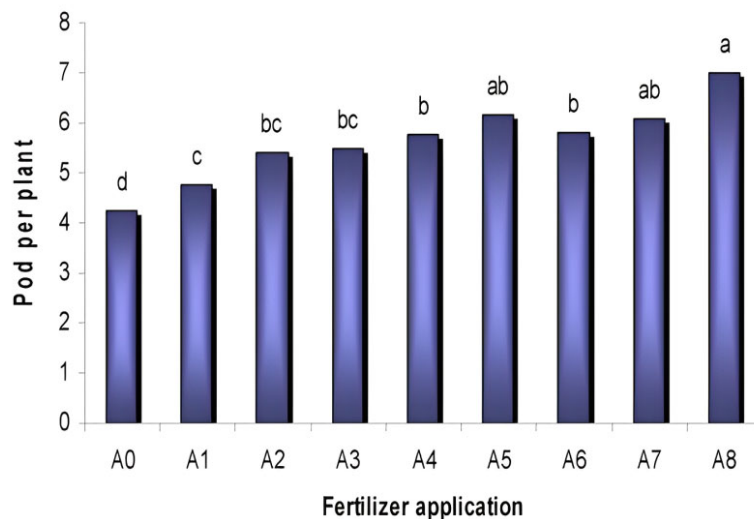


Figure 2. Effects of fertilizer application methods on bean pod number.

3.2. The number of seeds per pod

The number of seeds per pod was generally greater in the first year in comparison to the second year. But in both years, foliar application with micronutrients in the stem + flowering + pod stages (A_8) showed the highest levels of this trait. The multistage foliar application of the micronutrients showed better results than the single-stage soil application for both years. The reason is easier access at different stages of development. Foliar application of these elements showed the best results in the treatments with a stage after pod production. After producing pods, the access of the plant to these nutrients reinforced photosynthesis and increased the production of assimilate. This leads to a higher power source plant, and by regulating the relationship between the sink and the source, the plant was able to produce more flowers. Soil application with micronutrients in the first and second years led, respectively to 10.5% and 28.5% more seeds in the pods in comparison to the control group. With foliar application, the average increase for the first and second years was 11.7% and 39.7%, respectively (Table 4). Teixeira et al. (2004) and Ozaktan and Doymaz (2022) reported that the average maximum number of seeds per plant was 5.3, which resulted from the use of Zn and Mn, and this value was 17% higher compared to the control group.

3.3. 100 seed weight

The lowest 100 seed weight in both years was related to the control group (nonuse of fertilizers) among the other treatment groups. There was no significant difference between the other treatment groups in the second year, soil application, and multiple stage elements in the first year. The 100 seed weight in the second year was higher in all treatment groups in comparison to the first year because of higher seed production in the first year than in the second year of production. The application of micronutrients on average increased the 100 seed weight to 8.8% in the first year and 23% more than the control group (Table 4). Micronutrients increase the leaf area, leading to the creation of active photosynthesis with high assimilation ability. Increasing the amount of nitrogen increases the sink capacity (the number of endosperm cells and starch granules) and enzyme activity in seeds (invertase, sucrose synthase, and aspartate amino transaminase) (Costa et al., 2006). Sithaphanit et al. (2011) and Naser et al. (2022) reported that N has a direct role in developing seeds by increasing the levels of enzymes and enzyme activity, which increases the transfer and processing of incoming sucrose to seeds. Other researchers have stated that the availability of N before pollination is important for transferring assimilates and grain filling (Hirel et al., 2007; Yu et al., 2018). Mattas et al. (2011) reported that N fertilizer in several steps further affected the 1000 seed weight, and that the application of N fertilizer at 4 stages

led to a higher 1000 seed weight than its application in 2 stages. Moreover, increasing the use of N fertilizer increased the 1000 seed weight. Mattas et al. (2011) also examined the effect of different levels of N fertilizer on the weight of 1000 wheat seeds. The researchers found that for up to 150 kg/ha N fertilizer, the weight of 1000 wheat seeds increased, but with higher fertilizer weight, the weight of 1000 seeds decreased. Moreover, the use of N fertilizer in several stages led to a greater weight of 1000 wheat seeds, while with an increase in the amount of fertilizer, 1000 seed weight decreased. In this study, the application of N fertilizer to wheat grain several times led to 1000 seed weight.

3.4. Seed yield

The maximum seed yield by foliar application stem was related to elongation + flowering + pod of (A_8). There was no significant difference in the foliar applications at flowering + stem elongation (A_5) and flowering + pod (A_7), but there was a significant difference between these and the other treatments, foliar and soil application, and the no-treatment (control) group. Except for the mentioned three steps, the foliar application stages had no significant difference from the soil application stages (Figure 3). The additive effect of Zn foliar application in various stages of growth on yield per plant was greater than that of $MnSO_4$, but the soil application was significantly different from the others. Therefore, the soil application of these elements increased grain yield up to about 45 g/m² per unit area compared to the nonuse (control) group. The increase in $ZnSO_4$ foliar application at flowering + pod + stem elongation of (A_8) was about 200 g/m², and for $MnSO_4$, about 140 g/m². This increase could be attributed to the increased number of pods per plant and seeds per pod (Figure 3). The results of this study showed an increase in yield on two-year averages by taking three steps compared to the control and soil application of $ZnSO_4$ was 173 and 202 g/m², respectively. For $MnSO_4$, the average was 92 and 134 gr/m², respectively. This quantitative increase in grain production, due to the economic value of green beans, justifies the three-stage foliar application of the micronutrients. Yarnia et al. (2008) and Tabesh et al. (2020) reported that the application of $ZnSO_4$ in sugar beet root increased the yield. The increase was 4.9% in comparison to the control group. According to the researchers, the highest sugar yield (9.98 t/ha) was obtained with soil application of $MnSO_4$. Sharma et al. (2010) reported an increase in the Zn yield compared to the control group, regardless of the application method. This confirmed the results of the present experiment. Mohammed (2005) and Swierczynski and Antonowicz (2021) showed that foliar micronutrients of Fe, Zn, Cu, and Mn increased the tomato yield by up to 19.02%. Faiyad and Csatho (2005), in a greenhouse experiment, showed that by foliar application of Zn, the

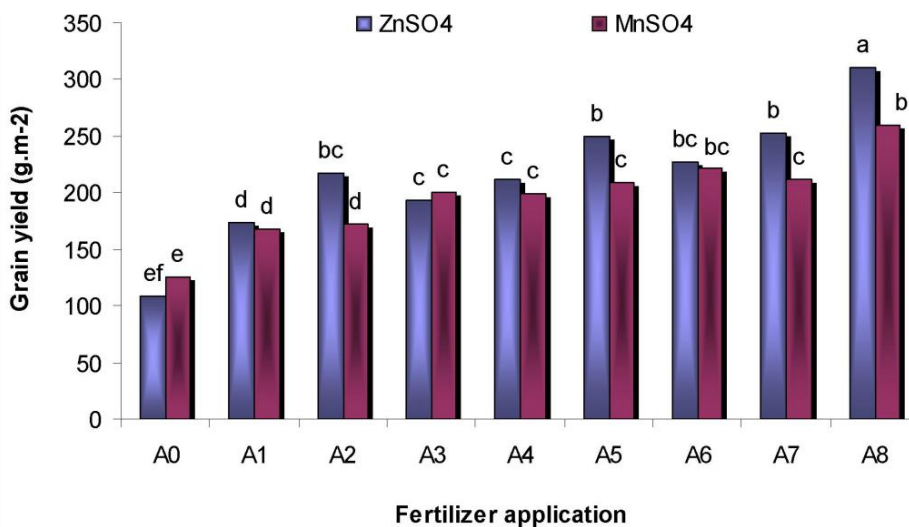


Figure 3. Effects of ZnSO₄ and MnSO₄ and application methods on bean grain yield.

yield increased at a rate of 36%. The application of Mn significantly increased the absorption of this element in the bean and corn (Fageria et al., 2007). Zn deficiency leads to loss of yield, crop growth, and the amount of bean protein (Kaya et al., 2005; Mahdieh et al., 2018). Zeidan et al. (2006) obtained similar results in lentil. They showed that Zn foliar application increased the weight of one thousand seeds with an increase in the amount of assimilation, leading to a yield increase. Salamatbakhsh et al. (2012) reported that foliar application of Zn in beans increased the seed yield. Ebrahim and Aly (2004) and Toor et al. (2020) reported that Zn foliar application increased the rate of chlorophyll a and b, the photosynthetic activity of photosystem II, and the initial assimilation rate in crops. According to these researchers, the changes in chlorophyll content could be related to the effects on the processes leading to the synthesis of chlorophyll (Ebrahimi et al., 2011). In a study on beans, Teixeira et al. (2004) and Afsahi et al., (2020) showed that the application of Zn and increasing the amount of chlorophyll in crops could raise the absorption of N and Mn, concluding that plants could produce more assimilates to increase yield. In the Teixeira et al. (2004) study, the number of seeds per pod was affected by foliar application of Mn and Zn; the effect of Zn was higher, and it increased bean yield up to 34%.

3.5. Harvest index

The harvest index was higher in the first year than in the second year. The reason was the higher yield of the crop in the second year than in the first year. In both years, the HI in terms of zinc sulfate was higher than manganese sulfate. ZnSO₄ increased all the seed traits more than MnSO₄, resulting in higher HI (Figure 4). Foliar application of micronutrient fertilizers in the growth stage promoted the expansion of photosynthesis organs, leading to the

transfer of more photosynthetic materials to the seeds. The increase in seed weight is directly linked to the increase in HI. Yarnia et al. (2008) showed that foliar application of MnSO₄ in sugar beet increased the harvest index to 18.35% compared to the control plant.

3.6. Micronutrients efficiency

The analysis of variance for the efficiency of the nutrients (Table 3) showed that the effect of foliar application was significant. The efficiency of the foliar application of micronutrients, especially in the pod production stage had the highest amount, and the lowest efficiency belonged to the soil application of fertilizer. All of the foliar application treatments were significantly higher than the soil application. In other words, when micronutrients were used in the form of soil, the plant was not able to use them efficiently in order to increase production (Figure 5). Slaton et al. (2001) and Mahmoud et al. (2022) reported that foliar application led to higher consumption and physiological efficiency than soil applications and that it increased the amount of Zn in the seed.

3.7. Zinc content in seed

The higher the amount of Zn in the immediate environment of the plant, the higher the amount of Zn in the plant will be. The Zn content of bean seeds increased with soil and foliar application of ZnSO₄.

Zn content increased with the increasing frequency of foliar application. The increase in grain Zn content was higher in the first year than in the second year. The greatest increase was respectively 216% and 124% for both years in the A₈ treatment group; foliar application of ZnSO₄ in both years increased the amount of these elements in seed more than soil application (Table 4). The presence of Zn and beans in the human diet is highly important, thus warranting much more attention and investigation. Zn

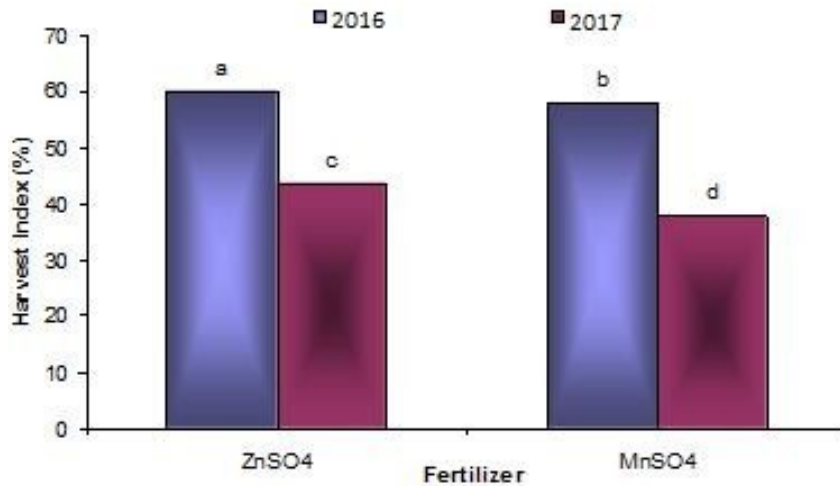


Figure 4. Effects of ZnSO₄ and MnSO₄ on bean harvest index.

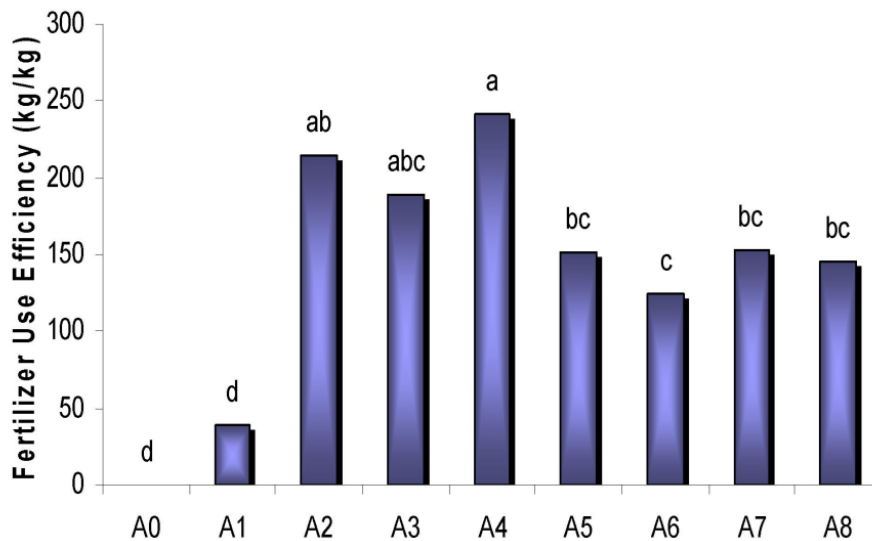


Figure 5. Effects of fertilizer application methods on bean fertilizer use efficiency.

has high mobility from the leaves to the roots, stems, and growing seeds (Rengel, 2001; Tena et al., 2020). Zn foliar application on the leaves leads to its higher accumulation in grains than soil application. Due to the calcareous nature of the soil in the experimental field, Zn is commonly used for adsorption in calcareous soils, and soil particles cannot easily move to the roots (Razzaq et al., 2013). The present study showed that the use of this micronutrient in the form of soil application was not suitable. Prasad et al. (2012) reported that foliar application of ZnSO₄ could lead to an increase in the size of grain crops. Khalifa et al. (2011) reported that foliar application of zinc fertilizer increased the amount of zinc in different parts of the plant. Nasiri et al. (2010) and Kumar et al. (2022) also

studied the effect of the foliar application of Fe and Zn on German chamomile, reporting that foliar application of the mentioned elements played an important role in both vegetative and reproductive growth of the plants and could increase the amount of crop. Haslet et al. (2001) also achieved similar results for wheat.

3.8. Mn content in seed

Mn content of bean seeds increased with soil and foliar application of Mn, and the content of Mn increased with increased frequency of foliar application. The Mn content of the bean was higher in the first year than in the second year. In both years, the amount of Mn in the beans was respectively 0.44 and 0.63 mg/g in the A₈ treatment. In the second year, no significant difference was observed in the

Table 4. Means comparison of studied traits and their changes compared to control in a field experiment (2016–2017).

| Change Compared to control (%) | Mn content in grain | | Change compared to control (%) | | Zinc content in grain | | Change compared to control (%) | | 100 kernel weight (g) | | Change compared to control (%) | | Number of pods per plant | | |
|--------------------------------|---------------------|-----------|--------------------------------|-------|-----------------------|--------|--------------------------------|--------|-----------------------|---------|--------------------------------|--------|--------------------------|--------|------|
| | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 |
| - | 0.42 d | 0.247 d | - | - | 116 e | 98 d | - | - | 30.87 b | 30.95c | - | - | 2.59 c | 3.32c | A0 |
| 33.3 | 0.56 ab | 0.257 cd | 44.9 | 76.5 | 168.1 d | 173 c | +19.63 | +4.43 | 36.93 a | 32.32b | +29.73 | 10.24+ | 3.36 b | 3.66b | A1 |
| 24.5 | 0.523 b | 0.317 bcd | 48.7 | 121.4 | 172.5 d | 217 bc | +21.44 | +10.18 | 37.49 a | 34.1a | +28.57 | 10.54+ | 3.33 b | 3.67b | A2 |
| 19.1 | 0.5 bc | 0.37 b | 73.3 | 96.9 | 201 bc | 193 bc | +21.25 | +5.98 | 37.43 a | 32.8b | +37.84 | -3.01 | 3.57 ab | 3.22c | A3 |
| 4.8 | 0.44 cd | 0.26 cd | 71.1 | 116.3 | 198.5 c | 212 bc | +20.86 | +6.37 | 37.31 a | 32.92b | +38.22 | +13.55 | 3.58 ab | 3.77ab | A4 |
| 43.6 | 0.603 a | 0.387 b | 79.8 | 155.1 | 208.5 bc | 250 ab | +23.45 | +8.17 | 38.11 a | 33.48ab | +38.22 | +6.93 | 3.58 ab | 3.55bc | A5 |
| 46.7 | 0.616 a | 0.33 bc | 90.6 | 131.6 | 221.1 b | 227 bc | +23.74 | +13.02 | 38.2 a | 34.98a | +42.09 | +11.45 | 3.68 ab | 3.70ab | A6 |
| 34.1 | 0.563 ab | 0.283 cd | 81.9 | 158.2 | 211 bc | 253 ab | +27.24 | +10.34 | 39.28 a | 34.15a | +38.61 | +10.54 | 3.59 ab | 3.67 b | A7 |
| 50 | 0.63 a | 0.437 a | 124.3 | 216.3 | 260.2 a | 310 a | +26.53 | +12.18 | 39.06 a | 34.72a | +54.05 | +17.17 | 3.99 a | 3.89 a | A8 |

Means in each column with the same letter have not significant difference at a 5% probability level.

amount of zinc in the seeds after several foliar applications. $MnSO_4$ application increased the rate of $MnSO_4$ in both years of the experiment more than its soil application. Mn washing is the most important factor of Mn loss in sandy soils with a high pH (Laszlo, 2008). Due to weekly irrigation and the sandy nature of the soil in the experimental area, the effectiveness of these two elements, especially foliar application, the plant growth and accumulation in the seed were normal. Talooth et al. (2006) also reported that Mn foliar application had positive effects on growth parameters, yield, as well as the content in mung beans. Concerning wheat, Zeidan et al. (2010) observed that $MnSO_4$ foliar application increased the Amaranth grain from 15.5 mg/kg to 74 mg/kg.

3.9. Germination test results

The ANOVA results indicated that the type of micronutrient consumption stage and their interactions were significant (Table 5). The results of the comparison showed significant differences in characteristics (Tables 6, 7, and 8). The maximum root length by Zn application was related to the soil consumption treatments and the Zn soil and foliar application treatments in the stem elongation + pod stages. The comparison of the effects of fertilizer on shoot length showed better performance for Zn than Mn (by 16.94%) (Table 6). The highest and lowest mean shoot lengths obtained from foliar application of flowering + pod and nonuse of it were 160.3 and 85.6 mm, respectively. In foliar treatments of seeds, longer seedling lengths resulted from the plants treated with Zn, but in soil treatments, the

use or nonuse of Mn led to longer seedling lengths. Due to the increased length of root and shoot and as a result of the use of micronutrients, the longer plant length was normal (Table 8).

The mean of root to shoot ratio in manganese fertilizer was significant, with a mean difference of 0.243 units. The maximum ratio of root to shoot was related to nonuse treatment, and the least was related to foliar application in flowering + pod production (Table 6). Foliar treatments, except for bolting and flowering stages, were not significantly different from each other, but they were far superior to soil application and control (Table 7). Seed treatments with Zn had 3.74% more germinated seeds than Mn treatments. The rate of germination in foliar application of flowering + stem elongation was more significant than other treatments, and the lowest amount was observed in the control group (Table 7). The maximum and minimum range of time speed germination were 2.61 and 2.12 days for control groups, and foliar application at stem elongation + flowering stages was owned by foliar application at stem elongation + flowering stage (Table 7). The shoot and plant height, seedling dry weight, and germination percentages were significantly higher for Zn consumption than for the use of Mn. The germination speed in foliar application was higher for soil application than in the control group. Thus, foliar application of Zn and Mg has a greater effect on seed vigor. Research has shown that foliar application of $ZnSO_4$ could increase the seed's Zn level from 15 mg/kg to 560 mg/kg. The

Table 5. Analysis of variance mean square for studied traits in germination experiment.

| S.O.V | df | MS | | | | | | | | |
|------------------------|----|-------------|--------------|-----------------|-------------------|---------------------|------------------------|------------------------|------------------|------------------------|
| | | Root length | Shoot length | Seedling length | Root: Shoot ratio | Seedling Dry weight | Germinated seed number | Germination percentage | Germination rate | Time speed germination |
| Fertilizer (A) | 1 | 2050.13 | 7634.36** | 17,603.14** | 1.07* | 0.66** | 5.02** | 223.06** | 1.61 | 0.013 |
| Application method (B) | 8 | 1141.61 | 5192.17** | 9573.78** | 0.657* | 0.31** | 0.75 | 33.36 | 58.98* | 0.16* |
| A×B | 8 | 1552.08* | 874.91 | 3925.85* | 0.19 | 0.05 | 0.45 | 20.07 | 32.61 | 0.1 |
| Error | 54 | 592.51 | 725.41 | 1514.20 | 0.24 | 0.035 | 0.63 | 28.21 | 24.74 | 0.066 |
| CV (%) | | 14.73 | 20.43 | 13.1 | 35.57 | 12.01 | 5.54 | 8.4 | 11.62 | 10.86 |

* and **: significant at 5% and 1%, respectively

Table 6. Means comparison of studied traits in germination experiment affected by fertilizer

| Fertilizer | Shoot length (mm) | Root: Shoot ratio | Seedling dry weight (g) | Germinated seed number | ination percentage (%) |
|-------------------|-------------------|-------------------|-------------------------|------------------------|------------------------|
| ZnSO ₄ | 142.13 a | 1.25 b | 0.17 a | 24.40 a | 97.59 a |
| MnSO ₄ | 121.53 b | 1.49 a | 0.15 b | 23.52 b | 94.07 b |

Table 7. Means comparison of studied traits in germination experiment affected by application method.

| | Shoot length (mm) | Root: Shoot ratio | Rate of germination (number/day) | Time speed germination (day) |
|---|-------------------|-------------------|----------------------------------|------------------------------|
| control (A ₀) | 6.58 e | 1.87 a | 38.27 c | 2.615 a |
| soil consumption (A ₁) | 1.11 de | 1.52 abc | 40.02 bc | 2.516 ab |
| stem elongation (A ₂) | 4.11 de | 1.59 ab | 43.19 abc | 2.345 abc |
| flowering stage (A ₃) | 9.12 cd | 1.57 abc | 43.53 abc | 2.324 abc |
| foliar application at: pod production (A ₄) | 14.4 ab | 1.2 bc | 43.67 abc | 2.346 abc |
| stem elongation + flowering (A ₅) | 14.6 bc | 1.32 bc | 47.43 a | 2.128 c |
| stem elongation + pod production (A ₆) | 16.3 a | 1.13 bc | 40.87 bc | 2.456 ab |
| flowering + pod production (A ₇) | 16.3 a | 1.20 c | 44.73 ab | 2.264 bc |
| flowering+ stem elongation + pod production (A ₈) | 15.2 a | 1.08 bc | 43.63 abc | 2.327 abc |

seedlings from these seeds were more vigorous, and the Zn concentration in the treated seedlings increased. The use of seeds with high concentrations of micronutrients increased the seedlings' resistance, leading to improved yield (Harris et al., 2007).

4. Conclusions

In general, due to the importance of seed quality in the production of desirable plant canopy, mother plant nutrition for seed production, especially nutrition by soil micronutrients based on soil tests, and the limitations of production, should be given due attention. In the present study, the foliar and soil application of ZnSO₄ and MnSO₄ led to higher quality and quantity of bean cv. Khomein. The increase in production was the result of increased yield. The highest efficiency of the ZnSO₄ and MnSO₄ was related to pod production and flowering stages efficiency of the ZnSO₄ and MnSO₄ was related to pod production and flowering stages, respectively. Meanwhile, the highest yield and its components for foliar application were achieved in

three phases. The increased yield in the foliar application in comparison to non use and soil application was respectively 173, and 202 g/m², and with the application of ZnSO₄, the proportions were respectively 92 and 134 g/m². The increased yield, compared with the costs incurred in the preparation and application of the fertilizers, was economical. Therefore, if there had been enough agricultural inputs, foliar application in three stages could have served as a suitable method for increasing the quality and quantity of the products. For both elements, the lowest efficiency in the use of fertilizers was related to soil application. Consumption of micronutrients in soil application means that by using of micronutrients in the form of soil consumption, the plant will not be able to use them efficiently for the qualitative and quantitative increase of the products. The low yield of this treatment was compared with the foliar application treatment. According to the results of the present study, using the micronutrients zinc and manganese sulfate caused higher germination in the beans in comparison to the seeds whose rootstock

Table 8. Means comparison of studied traits in germination experiment affected by application method and fertilizer.

| Treatment | Seedling dry weight (mm) | Root length (mm) | |
|-------------------|---|------------------|-----------|
| ZnSO ₄ | control (A ₀) | 222.3 f | 136 d |
| | soil consumption (A ₁) | 243.8 def | 140.8 cd |
| | stem elongation (A ₂) | 297.4 a-d | 159.9 bcd |
| | flowering stage (A ₃) | 331.2 ab | 187.9 ab |
| | pod production (A ₄) | 327.6 abc | 174.3 a-d |
| | stem elongation + flowering (A ₅) | 361.9 a | 207.8 a |
| | stem elongation + pod production (A ₆) | 336.3 ab | 171.2 a-d |
| | flowering + pod production (A ₇) | 334.7 ab | 171.3 a-d |
| | flowering+ stem elongation + pod production (A ₈) | 359.9 a | 186.6 ab |
| MnSO ₄ | control (A ₀) | 231.6 ef | 146.6 bcd |
| | soil consumption (A ₁) | 294.1 b-e | 179.9 a-d |
| | stem elongation (A ₂) | 247.6 d-f | 158.3 bcd |
| | flowering stage (A ₃) | 265.3 c-f | 164.8 bcd |
| | pod production (A ₄) | 294.1 b-e | 159.4 bcd |
| | stem elongation + flowering (A ₅) | 281.1 b-f | 154.1 bcd |
| | stem elongation + pod production (A ₆) | 330.4 ab | 180.7 abc |
| | flowering + pod production (A ₇) | 312.3 abc | 155.1 bcd |
| | flowering+ stem elongation + pod production (A ₈) | 277 b-f | 143.8 cd |

plants did not use micronutrients. Therefore, the use of the mentioned elements, particularly zinc sulfate, led to more and better production as well as greater vigor of the produced seeds for use in subsequent cultures.

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Compliance with ethical standards

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